| iGEM2014 – Microbiology – BMB – SDU | |
|---|---|
| **Title:** TSB transformation<br>**SOP number:** SOP0009_v01<br><br>**Version number:** 01 | **Date issued:** 2013.06.17<br><br>**Review date:** 2013.06.17<br><br>**Written by:** Patrick Rosendahl Andreassen |

## 1. Purpose

To transform E. coli cells with plasmid using TSB buffer

## 2. Area of application

*All E. coli cells*

## 3. Apparatus and equipment

| Apparatus/equipment | Location (Room number) | Check points | Criteria for approval/rejection |
|---|---|---|---|
| **Pipettes (p1000,200,10)** | Micro Storage | ● | |
| **Heating block** | Laboratory 1. Floor | ● | |
| **Ice** | Across V18-403b-2 | ● | |

## 4. Materials and reagents – their shelf life and risk labelling

| Name | Components | Supplier / Cat. # | Room (hallway storage) | Safety considerations |
|---|---|---|---|---|
| **Purple pipette tips** | | Contact lab-manager | Micro storage | |
| **Green pipette tips** | | Contact lab-manager | Micro storage | |
| **Blue pipette tips** | | Contact lab-manager | Micro storage | |
| **Fort. LB** | | The new Anne-mette | Autoclave room | |

| | | | | |
|---|---|---|---|---|
| **Polyethylene glycol (PEG) 3,350** | | Sigma Aldrich | Micro chemical room | |
| **Dimethyl sulfoxid (DMSO)** | | Sigma Aldrich | Micro chemical room | |
| **Magnesium chloride ($MgCl_2$) 1M** | | The new Anne-mette? | Autoclave room | |
| **Sterile filter (Pref. Blue)** | | Contact lab-manager | Micro storage | |
| **Plasmid** | | | | |
| **15mL falcon tube** | | Contact lab-manager | Micro storage | |
| **10mL syringe** | | Contact lab-manager | Micro storage | |
| **Long needle for syringe** | | Contact lab-manager | Micro storage | |

## 5. QC – Quality Control

Colony PCR on transformed cells using primers for the plasmid.

## 6. List of other SOPs relevant to this SOP

## 7. Environmental conditions required

## 8. Procedure

1. Preparation of E. coli culture:
   - 1.1. Add at least 5mL fort. LB (depending on amount of transformation to perform) to a bulb
   - 1.2. With a blue pipette tip add E. coli culture from agar plate to the LB media
   - 1.3. Grow culture to a OD600 of 0.3 to 0.5
2. Preparation of TSB buffer
   - 2.1. Add the following components to a 15mL falcon tube:
     - 2.1.1. PEG 3,350 1g
     - 2.1.2. DMSO 500µL
     - 2.1.3. $MgCl_2$ (1M) 200µL
     - 2.1.4. Fort. LB →10mL
   - 2.2. When everything is completely dissolved, transfer it to a new (sterile) falcon tube through

     a sterile filter using a syringe
3. TSB Transformation
   - 3.1. Spin 0.5-1.0mL culture for 5 min. at 4000 rpm.

3.2. Remove supernatant
3.3. Dissolve pellet in 200µL TSB buffer
3.4. Add plasmid (varying amount)
3.5. Keep at ice for 30 min.
3.6. Transfer directly to a heating block at 42°C for 2 min.
3.7. Add 1 mL fort. LB
3.8. Phenotypical expression at 37°C (0-2 hours)
3.9. Spin for 5 min. at 4000 rpm.
3.10. Remove most supernatant and dissolve pellet in the remaining supernatant (50-150µL)
3.11. Plate on agar plate with appropriate antibiotic

## 9. Waste handling

| Chemical name | Concentration | Type of waste (C, Z...) | Remarks |
|---|---|---|---|
| ON Culture | | Liquid bacterial waste | |
| Once use plastic | | GMO yellow waste | |

## 10. Time consumption

- Total-time 4-6 hours.
- Hands-on-time 45 min.

## 11. Scheme of development

| Date / Initials | Version No. | Description of changes |
|---|---|---|
| 13.06.18 / PRA | 01 | The SOP has been written |
| 13.06.18 / AK | 01 | The SOP has been approved |
| | | |
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## 12. Appendices